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challenge to structural biology. The folded domain structures for the scaffold protein PSD-95 were long known but such fragmentary knowledge lacks methods to assemble these pieces. We used single-molecule FRET studies with multi-parameter fluorescence detection [1] and filtered fluorescence correlation spectroscopy [2, 3] to describe the native state ensemble of this canonical scaffold protein. Our approach represents a solution to describing the flexibility in any multi-domain protein from nanoseconds to seconds. The five domains in PSD-95 partitioned into two independent supramodules. Intramolecular interactions were limited to neighboring domains, which did not interact without being tethered. Although MAGUK proteins are flexible, there are conformational preferences encoded in the primary sequence.

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295-Pos Board B75

Probing the Domain Motions of an Oligomeric Protein from Deep-Sea Hyperthermophile by Neutron Spin Echo

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Life is traditionally seen as being driven by energy from the sun, however deep sea organisms have no access to sunlight, so they depend on nutrients found in the dusty chemical deposits and hydrothermal fluids around the hydrothermal vent zones, where they live. In this study, we use neutron spin-echo spectroscopy (NSE) to measure the inter-domain motions of the inorganic pyrophosphatase (IPPase) enzyme derived from thermostable microorganisms. IPPase is of extreme interest for biophysical studies because of their inherent chemical and thermal stability and high temperature activity. It has a hexameric quaternary structure with a molecular mass of approximately 120kDa (each subunit is about 20kDa molecular weight), which is a large oligomeric molecular structure. Study of the slow inter-domain motions that occur in the protein is the key to understand why IPPase can perform catalytic activity at much higher temperature than normal enzymes, thus can adapt to the extreme environment present at the seabed [1-3]. NSE spectroscopy is able to probe these slow inter-domain motions directly in the time-domain, as has already been established in other studies[4,5]. The length and timescale of NSE are right in the ranges from sub-Angstrom and picoseconds to nanometers and several tens of nanoseconds and beyond. Distinguishable dynamical behavior found between two proteins reveals local flexibility and conformational substates unique to oligomeric structures. Our results greatly help understanding the relation between protein dynamics and their biological functions.

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296-Pos Board B76

The Mechanism of Population Shifting among Transition States of Adenylate Kinase

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The rearrangement of LID and NMP domains of adenylate kinase (ADK) in solution is important to ADK's catalytic function. To study the mechanism of the phosphotransferase reaction conduct by ADK, we applied a multi-basin structure-based (SMOG) coarse-grained model with targeted molecular dynamics (MD) protocols in the MD simulation of apo-ADK. The MD trajectories produce substantial ensembles that help to explore the transition states and pathways between the open and the closed states of ADK. We classified all observed states into 5 to 7 clusters using RMSD among structures as the metric for 'k-means' clustering. The comparisons of the x-ray solutionscattering pattern of ADK with that calculated from representative structures of each cluster were used to characterize the experimental ensembles under different solution conditions. Combined with the transfer rate between clusters, these analyses provide a framework for understanding the possible allosteric pathway underlying the catalytic function of this important enzyme.

297-Pos Board B77

Molecular Mechanism of Ruthenium and Gold Anticancer Agents in the Allosteric Regulation of the Histone Proteins of Chromatin

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Recently, transition-metal compounds have been proposed as effective chromatin-binding agents, with a modulatory action on gene expression and an important therapeutic potential for cancer treatment. In particular, Ru-based RAPTA (Ru(II), Arene, PTA = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1] decane) compounds appear to interfere with transcription, resulting in apoptosis. Besides, the gold-based compound Auranofin - a clinically approved antiarthritic drug - has shown significant antiproliferative properties in in vivo and in vitro models.

Here, we report the molecular mechanism of Auranofin and Rapta-T (T = Toluene) in the allosteric regulation of histone proteins of nucleosome core particles (NCP), the basic repeating unit of chromatin. High-resolution crystal structures indicate that Auranofin and Rapta-T bind at two sites of the NCP histone core that are ~35 Å apart. In spite of this, biochemical and quantitative ICP/MS experiments show a 3 fold increased binding of Auranofin when in combination with RAPTA-T, therefore suggesting a synergistic effect of the two compounds. Integrating these experimental results with long time scale molecular dynamics (MD) simulations, we are able to propose at the atomistic level the cooperative binding mechanism of Auranofin and Rapta-T, which is fundamental for the NCP allosteric regulation. Our results show that the presence of Rapta-T favors Auranofin binding, inducing a crucial conformational change in the C-terminus of histone H3. Overall, our results depict a novel mechanism for the allosteric regulation of chromatin and provide crucial insights on how transition-metal compounds could influence histone modifications and gene transcription. This provides mechanistic insights that could potentially lead to the discovery of new therapeutic strategies against cancer.

298-Pos Board B78

Characterizing Nucleotide Dependent Allostery in G-Proteins with Molecular Dynamics and Normal Mode Analysis

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G protein signaling mediates critical cellular processes ranging from excitability and motility to differentiation and proliferation. As such, mechanistic dissection of G proteins can inform our understanding of cellular function and dysfunction in many pathological conditions. It has now been established that G protein alpha subunits operate by oscillating between distinct GTP bound 'active' and hydrolysis induced 'inactive' conformations. However, the link between the crystallographically observed conformations and the fundamentally dynamic mechanisms involved in activation, effector interaction and their allosteric regulation remain unclear. Here we describe molecular dynamics (MD), ensemble normal mode analysis (eNMA) and novel bioinformatics analysis that characterize the dynamic coupling of allosteric sites. In particular, we identify distinct flexibilities and dynamic couplings that distinguish GTP-active, GDP-inactive and GDI-inhibitory states. Network analysis of dynamic couplings reveals a consistent bilobal dynamic domain substructure with state specific coupling strengths between distal functional sites. The GTP state is shown to have the strongest couplings, exhibiting an overall dynamical tightening of binding interfaces for receptor, nucleotide and effectors. Network path analysis delineates conserved residues critical for these couplings. Mutational simulations further support the functional relevance of the identified residuesour findings, with selected point mutations decoupling distal binding interfaces and leading to a spontaneous helical domain opening under nucleotide-bound conditions. These results are consistent with experiments indicating altered rates of nucleotide exchange. Finally, our studies reveal a high consistency between long-time MD and NMA applied to the available crystallographic ensemble. This supports the utility of new 'ensemble NMA' and correlation network analysis for rapidly characterizing nucleotide dependent functional dynamics and allosteric mechanisms. All methods utilized for structural dynamics and evolutionary analysis are made freely available in Bio3D version 2.1 http://thegrantlab.org/bio3d/.